

Application No. 09/724,265
Amendment dated July 30, 2003
Reply to Office Action of January 30, 2003

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. (CURRENTLY AMENDED) A method for the introduction of molecules into a cell, comprising:
 - (a) preparing a solution containing said molecules;
 - (b) supplying said solution for conversion to aerosol droplets at a flow rate of between about 1 μ l/minute to about 350 μ l/minute;
 - (c) producing aerosol droplets comprising said molecules;
 - (d) accelerating said aerosol droplets toward said cell; [[and]]
 - (e) impacting said cell with said accelerated aerosol droplets; and
 - (f) producing a cell containing said molecule.
2. (ORIGINAL) The method of claim 1, wherein said molecules are selected from the group consisting of carbohydrate, nucleotide sequence, plant growth regulator, peptide, and combinations thereof.
3. (WITHDRAWN) The method of claim 2, wherein said molecules comprise carbohydrate.
4. (ORIGINAL) The method of claim 2, wherein said molecules comprise nucleotide sequence.
5. (WITHDRAWN) The method of claim 2, wherein said molecules comprise plant growth regulator.
6. (WITHDRAWN) The method of claim 2, wherein said molecules comprise peptide.

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7. (ORIGINAL) The method of claim 1, wherein said cell is selected from the group consisting of plant cell, animal cell, and bacterial cell.
8. (ORIGINAL) The method of claim 7, wherein said cell is a plant cell.
9. (ORIGINAL) The method of claim 8, wherein said plant cell is a monocotyledonous plant cell.
10. (ORIGINAL) The method of claim 9, wherein said monocotyledonous plant cell is a corn cell.
11. (ORIGINAL) The method of claim 8, wherein said plant cell is dicotyledonous plant cell.
12. (ORIGINAL) The method of claim 11, wherein said dicotyledonous cell is a soybean cell.
13. (ORIGINAL) The method of claim 1, wherein said aerosol droplets which impact said cell comprise aerosol droplets which are less than 0.1 micron in diameter.
14. (ORIGINAL) The method of claim 1, wherein said aerosol droplets are continuously targeted toward said cell.
15. (ORIGINAL) The method of claim 1, further comprising the placement of said cell on a target surface the linear and rotational movement of which can be separately controlled.

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16. (ORIGINAL) The method of claim 4, wherein said nucleotide sequence comprises a vector.
17. (CANCELED)
18. (ORIGINAL) The method of claim 1, wherein said aerosol droplets are produced by a microflow nebulizer.
19. (ORIGINAL) The method of claim 8, wherein said molecules comprise nucleotide sequences, and said method further comprises transforming said plant cell with said nucleotide sequences and regenerating a transgenic plant from said transformed plant cell.
20. (ORIGINAL) The method of claim 19, further comprising obtaining progeny of said transgenic plant, wherein said progeny comprises said nucleotide sequence.
21. (WITHDRAWN) The transgenic plant produced by the method of claim 19, wherein said transgenic plant is a monocot.
22. (WITHDRAWN) The transgenic monocot plant of claim 21, wherein said monocot is a corn plant.
23. (WITHDRAWN) The transgenic plant produced by the method of claim 19, wherein said transgenic plant is a dicot.
24. (WITHDRAWN) The transgenic dicot plant of claim 23, wherein said dicot is a soybean plant.

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25. (WITHDRAWN) The progeny of the transgenic plant of claim 21.
26. (WITHDRAWN) The progeny of the transgenic plant of claim 22.
27. (WITHDRAWN) The progeny of the transgenic plant of claim 23.
28. (WITHDRAWN) The progeny of the transgenic plant of claim 24.
29. (WITHDRAWN) Cells obtained from the progeny of claim 20.
30. (WITHDRAWN) A method of enhancing embryogenic callus production from embryos of elite soybean lines which comprises culturing said embryos on a medium comprising phytic acid, wherein the concentration of said phytic acid is between about 1 gm/l to less than 3000 mg/l.
31. (WITHDRAWN) A method of enhancing embryogenic callus production from embryos of elite soybean lines which comprises culturing said embryos on a medium comprising coconut water, wherein the concentration of said coconut water is between about 3% and about 6% by volume of said medium.
32. (WITHDRAWN) The method of claim 31, wherein said medium further comprises myoinositol and wherein the concentration of said myoinositol is about 1g/l to about 10g/l.
33. (WITHDRAWN) The method of claim 32, wherein said medium further comprises inorganic phosphorous.

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34. (WITHDRAWN) The method of claim 33, wherein said inorganic phosphorous is in the form of KH_2PO_4 and wherein the concentration of said KH_2PO_4 is between about 500 mg/l and about 1000 mg/l.
35. (WITHDRAWN) The method of claim 32, wherein said medium further comprises phytic acid and wherein the concentration of said phytic acid is about 1mg/l to less than about 3000 mg/l.
36. (WITHDRAWN) The method of claim 33, wherein said medium further comprises phytic acid and wherein the concentration of said phytic acid is about 1mg/l to less than about 3000mg/l.
37. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean embryo, prior to impact on a medium comprising phytic acid, wherein the concentration of phytic acid is between about 1 mg/l to less than 3000 mg/l.
38. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean embryo, prior to impact on a medium comprising coconut water, wherein the concentration of coconut water is between about 3% and about 6% by volume of said medium.
39. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean embryo, prior to impact on a medium comprising coconut water, wherein the concentration of coconut water is between about 3% and about 6% by volume of said medium, and myoinositol, wherein the concentration of myoinositol is between about 1 g/l to about 10g/l.

40. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean embryo, prior to impact on a medium comprising coconut water, wherein the concentration of coconut water is between about 3% and about 6% by volume of said medium, myoinositol, wherein the concentration of myoinositol is between about 1 g/l to about 10g/l, and inorganic phosphorous, wherein the concentration of inorganic phosphorous is between about 500 mg/l and about 1000 mg/l.
41. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean embryo, prior to impact on a medium comprising coconut water, wherein the concentration of coconut water is between about 3% and about 6% by volume of said medium, myoinositol, wherein the concentration of myoinositol is between about 1 g/l to about 10g/l, and phytic acid, wherein the concentration of phytic acid is between about 1 mg/l to less than 3000 mg/l
42. (WITHDRAWN) The method of claim 12, further comprising culturing said soybean cell, wherein said cell is from a somatic soybean cell, prior to impact on a medium comprising coconut water, wherein the concentration of coconut water is between about 3% and about 6% by volume of said medium; myoinositol, wherein the concentration of myoinositol is between about 1 g/l to about 10g/l; inorganic phosphorous wherein the concentration of inorganic phosphorous is between about 500 mg/l and about 1000 mg/l, and phytic acid, wherein the concentration of phytic acid is between about 1 mg/l to less than 3000 mg/l.

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43. (WITHDRAWN) The method of claim 12, further comprising exposing the soybean cell to about 1gm/l phytic acid for about 3 to about 10 days before impacting said soybean cell.

44. (WITHDRAWN) An aerosol beam apparatus, comprising:
 - a vacuum chamber having a nozzle and adapted to contain a target to be impacted;
 - an entrainment housing having an interior communicating with said vacuum chamber;
 - a pressurized gas supply;
 - a microflow nebulizer located wholly or partially within said entrainment housing and having a nebulizer orifice;
 - a sample material supply conduit communicating with said microflow nebulizer and an sample material supply for supplying sample material;
 - a nebulizer conduit communicating with said pressurized gas source and the microflow nebulizer; and
 - an entrainment tube communicating with the pressurized gas source and said interior of said entrainment housing;
 - wherein a pressurized gas from the pressurized gas supply travels through said nebulizer conduit to the microflow nebulizer and said sample material is carried by the pressurized gas through said nebulizer orifice, whereupon the pressurized gas and the sample material travel through said interior of the entrainment housing and enter the vacuum chamber through a nozzle and impact said target while the pressurized gas also travels through the interior of the entrainment tube to the entrainment housing and a resulting pressurized entrainment gas flows in a guiding manner through the entrainment housing substantially parallel to and around said pressurized gas and the sample material.

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45. (WITHDRAWN) The aerosol beam apparatus of claim 44, further comprising a syringe pump connected to said sample material supply conduit.
46. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said vacuum chamber is evacuated to a partial vacuum of about 26 to about 30 inches of Mercury.
47. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said vacuum chamber further includes a movable stage for supporting said target.
48. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said pressurized gas supply is a pressurized helium supply.
49. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said pressurized gas supply further includes a filter.
50. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said pressurized gas supply further includes at least one pressure regulator.
51. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said pressurized gas supply further includes a pressure regulator before the nebulizer conduit and a pressure regulator before said entrainment tube.
52. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said entrainment housing further includes a nucleospot.

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53. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said entrainment housing further includes a temperature controller for controlling a gas temperature in said entrainment housing.
54. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said entrainment housing further includes a temperature controller that maintains a gas temperature in said entrainment housing in a range of about 32 degrees to about 80 degrees Centigrade.
55. (WITHDRAWN) The aerosol beam apparatus of claim 45, wherein said nozzle has an orifice with a diameter of about 200 microns to about 500 microns.
56. (ORIGINAL) The method of claim 10, wherein the said flow rate is between about 1 $\mu\text{l}/\text{minute}$ and about 200 $\mu\text{l}/\text{minute}$.
57. (ORIGINAL) The method of claim 56, wherein the said flow rate is between about 4 $\mu\text{l}/\text{minute}$ and about 50 $\mu\text{l}/\text{minute}$.
58. (ORIGINAL) The method of claim 57, wherein the said flow rate is between about 8 $\mu\text{l}/\text{minute}$ and about 50 $\mu\text{l}/\text{minute}$.
59. (ORIGINAL) The method of claim 12, wherein the said flow rate is between about 1 $\mu\text{l}/\text{minute}$ and about 200 $\mu\text{l}/\text{minute}$.
60. (ORIGINAL) The method of claim 59, wherein the said flow rate is between about 4 $\mu\text{l}/\text{minute}$ and about 50 $\mu\text{l}/\text{minute}$.

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61. (ORIGINAL) The method of claim 60, wherein the said flow rate is between about 8 μ l/minute and about 50 μ l/minute.
62. (WITHDRAWN) The method of claim 7, wherein said cell is a bacterial cell.
63. (ORIGINAL) The method of claim 60, wherein the said flow rate is between about 1 μ l/minute and about 200 μ l/minute.
64. (ORIGINAL) The method of claim 63, wherein the said flow rate is between about 4 μ l/minute and about 50 μ l/minute.
65. (ORIGINAL) The method of claim 63, wherein the said flow rate is between about 8 μ l/minute and about 17 μ l/minute.